U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-296 902

Plutonium Uptake by Plants Grown in Solution Culture

(U.S.) Environmental Monitoring and Support Lab, Las Vegas, NV

Aug 78

TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/3-78-081	2.
3. PB296902	
4. TITLE AND SUBTITLE: PLUTONIUM UPTAKE BY PLANTS GROWN IN SOLUTION CULTURE	5. REPORT DATE: August 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): James C. McFarlane, Allan R. Batterman, and Kenneth W. Brown	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Las Vegas, Nevada 89114	10. PROGRAM ELEMENT NO. 1FA628
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: U.S. Environmental Protection Agency--Las Vegas, NV, Office of Research and Development, Environmental Monitoring and Support Laboratory, Las Vegas, Nevada 89114	13. TYPE OF REPORT AND PERIOD COVERED: Final
	14. SPONSORING AGENCY CODE: EPA/600/07
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution Radiobiology Plutonium		13B 06R 18B

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 20
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: A02

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

United States
Environmental Protection
Agency

Environmental Monitoring
and Support Laboratory
P.O. Box 15027
Las Vegas NV 89114

EPA-600/3-78-081
August 1978

PB 296902

Research and Development

EPA

Ecological Research Series

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EPA-600/3-78-081
August 1978

PLUTONIUM UPTAKE BY PLANTS GROWN IN SOLUTION CULTURE

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FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory—Las Vegas contributes to the formation and enhancement of a sound monitoring data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report describes some of the characteristics of plutonium bonding to plant roots. The work was undertaken to learn about the plant-plutonium interactions which determine the contamination of plants, the possible use of plants as plutonium scavengers and their use as aquatic integrators for plutonium monitoring. This information will be of value to those concerned with monitoring plutonium in aquatic and marine environments. It will also be interesting to those studying the uptake and distribution of plutonium in plants. For further information on this subject contact the Pollutant Pathways Branch of the Monitoring Systems Research and Development Division.

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INTRODUCTION

The distribution of plutonium (Pu) in terrestrial and aquatic environments has resulted primarily from the fallout of nuclear debris. In 1968, Langham (1968) estimated the amount of Pu in the world environment from all sources was about 500 kilocuries; the primary source was fallout from nuclear atmospheric testing. Although most of the Pu has been distributed as worldwide fallout, the concentration resulting from this source is very low. Primary research emphasis has therefore been on the distribution of Pu from accidental releases and most work has emphasized terrestrial systems. According to Noshkin (1972), "We know very little about the behavior of plutonium and relatively nothing of the behavior of other transuranics in the ocean and far less of the behavior and fate of these elements in fresh-water environments." The experiments presented here were conducted to provide information regarding Pu absorption and adsorption in plants rooted in solutions. Comparisons are made between terrestrial and aquatic root environments in regards to Pu uptake and deposition in foliar tissue.

SUMMARY

Plants grown in aquatic systems were shown to rapidly accumulate large amounts of plutonium, about 40% of which was removed by washing. Detergent removed debris, most of which consisted of particles larger than 0.8 micrometers (μm). After removing a portion of the bound Pu by rinsing in DTPA, additional Pu was removed by a citric acid rinse. This implies that more than one type of Pu binding to plant roots exist, or that more than one chemical form of Pu are present. The high Pu concentration on plant roots did not facilitate uptake and translocation to aerial portions of the plant: discrimination ratios were similar to those typically found in terrestrial studies. Plants with filamentous root systems are suggested as possible scavengers for Pu in aquatic systems.

It is recommended that pilot studies be conducted to evaluate the use of plants in accident cleanup technology. Consideration should be given to the use of plants as being the best samples to represent Pu contamination in marine and fresh water environments.

METHODS

The uptake of Pu from solution cultures was measured for three different plants: Medicago, Lemna, and Typha. Medicago sativa (alfalfa) plants were grown in a glasshouse in hydroponic solution (Berry 1971); leaves and stems were cut when the plants were three-fourths flowering. Approximately 5 to 6 weeks were required between cuttings. At the time of each cutting, prior to Pu amendment, the roots were examined and dead roots were pruned. The plants were producing 30 to 40 grams (g) of oven-dried alfalfa per plant per cutting. The experiment consisted of two treatment groups. The acute treatments were accomplished by injecting 20 microcuries (μCi) of plutonium-238 nitrate [$^{238}Pu(NO_3)_4$] in 1.0N nitric acid (HNO_3) into each of four 7-liter containers filled with 1/2-strength Hoagland's nutrient solution. The solution was mixed, the pH was adjusted to 5.5 by addition of potassium hydroxide (KOH), and then the plants were returned to the solution. The chronic treatments were created by adding the same amount (20 μCi/pot) of Pu by continuous injection [0.25 milliliters per hour (ml/h)] over the entire 43-day growth period. The pH of the culture solution was tested weekly; it did not vary significantly from its original 5.5 and was therefore not adjusted. The solution volume in each container was brought to a constant level daily by the addition of 1/4-strength nutrient solution. Samples were collected from the containers and from the Pu stock solution at 3-day intervals and analyzed for ^{238}Pu content by liquid scintillation counting. At the termination of the chronic injection period, all plants were collected and leaves and roots analyzed for Pu. The roots were washed in a dilute nonionic detergent and then rinsed in 0.01N HNO_3. Neutralized nitric acid digests of all tissues were analyzed for Pu concentration by liquid scintillation counting.

Lemna minor (duckweed) was grown in 3 liters of 1/2-strength modified Hoagland's solution (Berry 1971) contained in each of eight 19-liter plastic containers. Constant agitation was accomplished by a magnetic stirring rod placed in the bottom of each container. Solution temperature was controlled to 25 ± 1°C by a heat-exchanging coil which circulated temperature-controlled water in each container from a temperature-control bath. The Hoagland's solution in each of four containers received 1.0 μCi of ^{238}Pu in 1.0 ml of stock solution. The other four received a chronic exposure in which the same amount of ^{238}Pu was added over a 30-day period.

An approximation of Pu concentration in Lemna tissues was obtained by adding Lemna plants (approximately 0.5 g wet weight) to tared scintillation vials. Twelve ml of scintillation cocktail and 9 ml of H_2O were then added. The solution was shaken, allowed to settle for 2 days, and counted on the liquid scintillation counter. An internal standard of ^{238}Pu was then added to obtain the efficiency for each vial. Efficiencies determined in this

manner were 0.99 ± 0.02 and did not vary significantly from the standard prepared in distilled water. Although this analytical technique does not allow for the complete dissolution of the sample, all of the water and most of the organic components were dissolved into the scintillation cocktail.

Typha angustifolia (cattail) was grown in the glasshouse in a recirculating hydroponic system. The system consisted of seven 19-liter polyethylene containers connected with polyvinyl chloride (PVC) pipe 4.3 centimeters (cm) inside diameter, which were placed on stair-like supports. Each container was 2.5 cm lower than the preceding one and the overflow pipe emptied at the bottom of the adjacent container thus facilitating solution mixing. The solution was pumped from the seventh container to the first at a flow rate of 1.5 liters per minute. The amount of solution in the system was regulated by a float switch which actuated the pump in the solution reservoir. By monitoring the solution level in the reservoir, the evapotranspiration rate of the system was determined.

At harvest, there was approximately 18 kilograms of plant material distributed about equally between the six segments of this system. Roots made up 63% of the dry plant mass and leaves the remaining 37%. The root volume was estimated to be 2.4 liters per container.

Plutonium nitrate (0.9 μCi) in 0.1N HNO_3 was injected into the hydroponic culture system at a constant rate over a 24-hour period. The delivery nozzle, a stainless steel 26-gauge tuberculin needle, yielded a nozzle velocity of 2.2 cm per minute (cm/min). This was rapid compared to ionic diffusion; therefore, no significant diffusional movement occurred up the nozzle. This precluded the possibility of Pu precipitation in the nozzle caused by a changing pH or contact with other ions present in the culture solution. Dispersion into the nutrient media was ensured by the continuous and rapid flow of culture solution past the nozzle (approximately 23 cm/min).

Solution samples were periodically collected from each container and the solution analyzed for Pu concentration by liquid scintillation counting. A small plastic tube extending into the middle of each container was connected at the top to a large diameter hypodermic needle. At each sampling period, a syringe was attached to the needle and repeatedly filled and emptied to ensure a representative sample. Eight ml of solution were drawn into the syringe and dispensed directly into a scintillation vial containing 12 ml of scintillation cocktail (Lieberman and Moghissi, 1970). Using the total energy band of the liquid scintillation counter, backgrounds were on the order of 30 counts per minute (cpm); the efficiency ranged from 1.00 to 1.05. Radioactivity due to plutonium-238-contaminated plant debris in the culture solution was estimated by counting replicate solution samples before and after filtration through 0.8, 0.45, 0.22, and 0.01-micrometer (μm) filters.

One month after the Pu addition, leaf and root samples were collected to evaluate the uptake and distribution of Pu in these tissues. Roots were cut off at the crown and placed into a cheesecloth basket. Leaves were divided into submerged leaves, old leaves, and new leaves. The leaf tissue was dried and weighed; subsamples were sent to Eberline Laboratories, Inc.,

for ^{238}Pu analysis. The roots were washed in a series of four different solutions. This was accomplished by moving the cheesecloth container up and down repeatedly in 4 liters of each solution for a period of 1 minute. The solutions were: distilled water in which 0.5 ml of a non-ionic detergent had been added, 0.01M DTPA (diethylenetriaminepentacetate), 0.01M citric acid, and 0.1M calcium chloride. Washings were repeated two or three times with fresh aliquots of the washing solution, and the roots were allowed to drain for 2 minutes between washes. The wash solutions were analyzed for ^{238}Pu by liquid scintillation counting after filtering. The Pu activity on each filter was determined by counting the alpha particle associated with ^{238}Pu decay on a 2-inch zinc sulfide scintillation crystal attached to a 2-inch photomultiplier (Thrall et al., 1977).

The calculated salt concentration of the nutrient culture was 0.008M. This corresponded to an electrical conductivity (E.C.) of 3.7 millimhos (mmho). Electrical conductivity was monitored throughout the growth of the Typha plants and kept constant by the addition of nutrient solution or distilled water. Make-up solution was added through the recirculating system and measurements of E.C. in each container showed that no nutrient gradient existed in the system.

RESULTS

Alfalfa plants growing in hydroponic solution were treated by adding $^{238}Pu(NO_3)_4$ to the root culture. The Pu concentration in the solution of the chronic exposure rose for the first 10 days to approximately 23 nCi/ml and remained at that concentration throughout the test period. The concentration of Pu in the acute exposure fell rapidly to an apparent equilibrium concentration of approximately 0.01 nCi/ml. Ten days after Pu exposure started and the plants were approximately two-thirds mature, the tops were cut and analyzed. Leaf concentrations were 0.10 ± 0.02 and 0.80 ± 0.40 nCi/g dry for the chronic and acute exposures, respectively. Thirty-three days later all plants were harvested and the leaf concentrations were then 28.0 ± 4.0 and 2.4 ± 0.4 nCi/g dry for the chronic and acute exposures, respectively.

In both the acute and chronic exposures more than 98% of the Pu injected was associated with the root tissue and less than 0.1% was found in the top of the plants. The ratio of Pu concentrations in the leaves to that in the roots after the final harvest was 3.0×10^{-4} and 1.7×10^{-4} for the chronic and acute exposures, respectively. Repeated washings of the roots in weak detergent or in 0.01M HNO_3 released only very small amounts of Pu from the roots. This suggests that the Pu was bound to the root through chemical intermolecular forces other than surface adhesion.

Following the acute injections of Pu into the Lemna containers the ^{238}Pu concentration decreased exponentially over the first 10 days, reaching an apparent equilibrium concentration of approximately 10 pCi/ml. Pu

administered as a chronic injection rose to 25 pCi/ml during the first 5 days and remained at that concentration throughout the rest of the test.

At the conclusion of the 33-day chronic exposure, the Lemna plants were collected from the surface of the treatment solutions with a fine-mesh plastic screen. The plants were placed into a cheesecloth envelope and washed sequentially in various solutions. The data in Table 1 show the concentration of Pu in the wash solutions.

TABLE 1. PLUTONIUM-238 CONCENTRATION IN WASH SOLUTIONS IN WHICH LEMNA PLANTS WERE SEQUENTIALLY WASHED IN WATER 0.01M DTPA AND 0.01M CITRIC ACID

WASHING SOLUTION		ACUTE Pu ADDITION (pCi/ml)	CHRONIC Pu ADDITION (pCi/ml)
H_2O	1	14 ± 3	3 ± 2
	2	20 ± 3	6 ± 2
	3	18 ± 4	8 ± 2
DTPA	1	89 ± 3	90 ± 11
	2	27 ± 2	22 ± 2
CITRATE	1	112 ± 3	48 ± 8
	2	44 ± 4	27 ± 7

The initial washing in all cases was water containing a small amount of nonionic detergent. This was done in an effort to remove any particles adhering to the roots by surface tension. In both cases, the concentration found in the water was quite low compared to the concentration in the chelating agents. Filtration of these solutions through a 0.8-μm filter removed only small amounts of the Pu from solution. The first washing with DTPA caused a large elution of Pu from the Lemna plants. The second washing released considerably smaller amounts of Pu; however, a subsequent washing with the citric acid solution released a large fraction of Pu from the tissues. While more Pu was removed from the acutely dosed plant tissues by the citrate washing, the pattern of Pu release was similar to that obtained in the chronically dosed plants. Comparison between washed and unwashed plants showed that 5 ± 3% of the original plutonium remained on the washed plants in

the acutely dosed treatment while 14 ± 6% remained on the chronically dosed plants. It is not presently apparent what caused the difference in binding.

Prior to treating Typha plants with Pu a similar system without plants was studied. Plutonium nitrate (50 μCi) was injected into the system at a constant rate over a 24-hour period. The peak concentration reached at the end of the injection was 425 pCi/ml. This represented 90% of the theoretical peak assuming that no precipitation or adsorption had occurred. Following a similar injection into the system containing Typha plants the peak concentration was only 52% of the theoretical. The difference was due to rapid adsorption of Pu to the plant roots. After the injection was completed the Pu concentration in both the Typha and control tests decreased with time finally reaching an apparent equilibrium of approximately 100 pCi/ml.

Because of the roots, the initial decreasing rate of the Pu from the Typha test solution was significantly faster than in the control (Figure 1). However, after the first few days, the slopes were similar and the loss rates seemed to be independent of the presence of the Typha roots and submerged leaves.

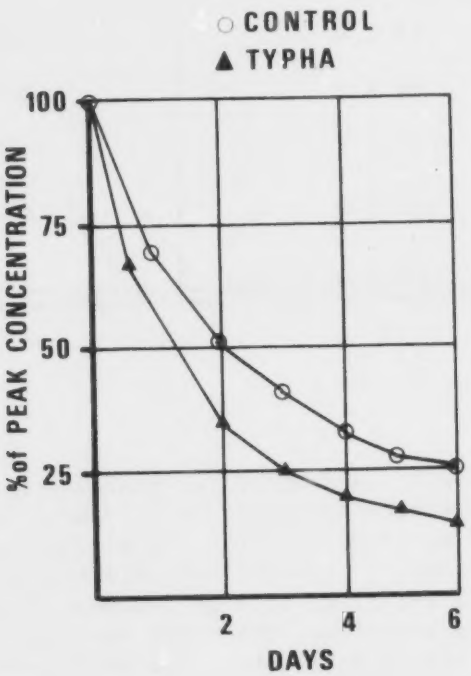

Figure 1. Plutonium loss from solution with and without Typha plants. ^{238}Pu activities are expressed as a % of the peak concentration and day zero coincides with the termination of injection.

Filtration of the control solution showed that about 90% of the Pu was associated with particles in the hydroponic solution. The results of the filter-filtrate tests are shown in Table 2. Most of the Pu labeled particles were larger than 0.8 μm and only about 10% of the Pu was either in solution or associated with particles smaller than 0.01 μm. In the Typha test Pu was not found in such abundance on free debris, but was mostly found on plant roots.

TABLE 2. PLUTONIUM ACTIVITY ASSOCIATED WITH DIFFERENT-SIZED PARTICLES IN CULTURE TANKS WITHOUT PLANTS

Plutonium-238 concentration remaining in solution after passing through various-sized filters. Activity expressed as percent of the unfiltered solution.

Filter Pore Size	Serial Filtration	Parallel Filtration	Control*
No Filter	100 ± 5.0	100 ± 1.6	100 ± 1.3
0.80 μm	30 ± 0.3	32 ± 1.2	99 ± 0.6
0.45 μm	25 ± 0.3	21 ± 1.3	100 ± 0.4
0.22 μm	11 ± 0.3	8 ± 0.9	99 ± 0.0
0.01 μm	---	8 ± 0.2	100 ± 0.0

± = 1 standard deviation.

* = Control consisted of sterilized culture solution in which no algae growth occurred and no dust was allowed.

At harvest the plants were separated into several subsamples for analysis. The plutonium-238 concentration in each of these subsamples is shown in Table 3. The designation "young leaves" refers to those leaves newly emerged; they were approximately half the length of the most fully matured leaves. Five-centimeter samples were taken 15 cm from the terminal end of the leaf. The submerged leaf samples consisted of cross sections of all leaf tissue, approximately 5 cm long. Young roots were those newly developed and still white; all other root material was considered to be old.

The evapotranspiration rate of the entire Typha system was determined throughout the Pu study. Water loss rates increased as the plants became larger. During the last week of the study, the water loss rates were 58 ± 5 milliliters per minute (ml/min) in the light and 8 ± 2 ml/min in the dark. Based on leaf area, transpirational water losses from young and old leaves were not significantly different. This was determined by the measurement of diffusive resistance to water vapor loss which was 16.8 ± 1.5 and 17.0 ± 20 seconds per cm on a clear day at noon for old and young leaves.

TABLE 3. PLUTONIUM-238 DISTRIBUTION IN VARIOUS TYPHA TISSUES 30 DAYS AFTER CONTAMINATING THE HYDROPONIC CULTURE WITH PLUTONIUM NITRATE

Tissue	nCi/g*
Young leaves	1.5 ± 0.5
Old leaves	0.5 ± 0.2
Submerged leaves	22 ± 8
Young roots	596 ± 199
Old roots	2389 ± 714

* = Concentration is based on tissue dry weight.

± = 1 standard deviation.

The difference between Pu concentration in young and old leaves (Table 3) is opposite in direction to that expected if deposition were a passive phenomenon associated with water movement. The young leaves developed subsequent to Pu treatment, and because of their size and age the total amount of water which passed through the xylem system was significantly smaller than through the older leaves. Also, the high Pu concentrations during and immediately after Pu injection were concurrent with old leaf transpiration, but occurred at a time when young leaves were embryonic. A calculation using the total dry weight of the leaves and the transpiration rates indicated that 60 ml of water per gram of dry leaf matter per day passed through the leaf. Considering that the Pu reached a peak concentration in solution of 4.5 nCi/ml and decreased to an average of 0.1 nCi/ml during the last 10 days of this experiment, it is obvious that Pu uptake was not coincident with the passive transport of water. Therefore, higher Pu concentrations found in the young leaves are thought to represent the continued presence of Pu throughout growth and development of leaf tissue. This also suggests that Pu accumulation in leaves may be associated with the movement of organics transported in the phloem. Submerged Typha leaves are held together in a sheath and only the outer cuticles of the outer leaves are in actual contact with the water. Therefore, the high activity on the submerged leaves is thought to represent only surface contamination of the outer sheath and it is lower than the root activity because of the difference in the surface area to mass ratio.

The young roots developed after the Pu addition and were therefore exposed to lower levels than the old roots which were present at the beginning of the experiment. This accounts for the difference in Pu content. The large variability associated with these means represents a lack of precision in separating the two root types.

Samples were collected from the bottom of alternate pots with a large syringe and an agitator next to the bottom of the pot. This was done in an effort to ascertain differences in particle-size distribution in the debris found in the settlings. Aliquots of each solution were filtered through 0.8, 0.45, and 0.22-μm filters. The data from these tests are presented in Figures 2 and 3.

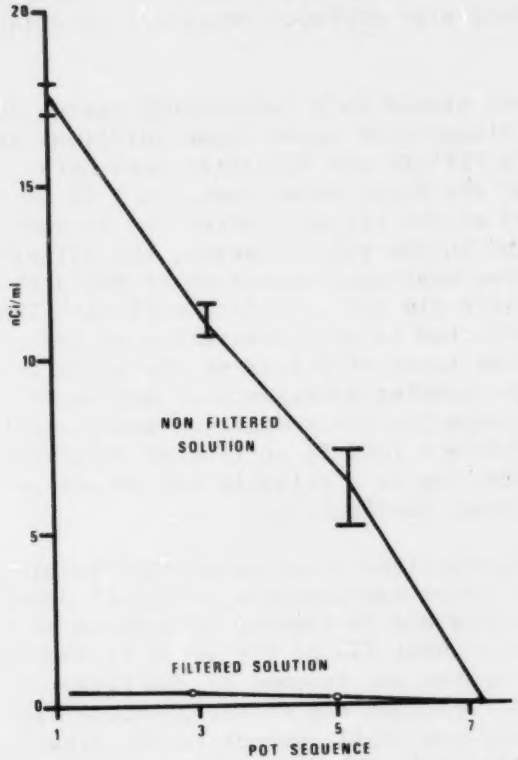

Figure 2. Plutonium contaminated sediments in alternate buckets. Confidence brackets represent ± standard deviation.

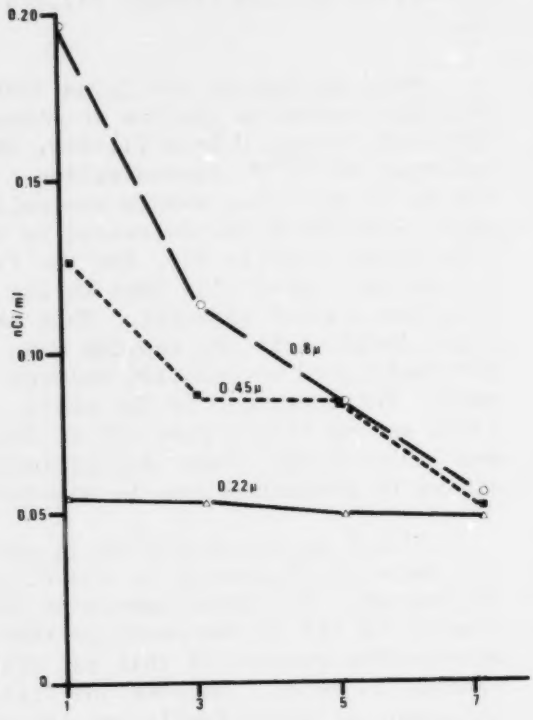

Figure 3. Plutonium in solution after sediments were removed by filtration. This is an expansion and separation of the components which made up the line shown in Figure 2 (filtered solution).

A pronounced gradient occurred in the Pu concentration of debris in the direction of solution flow. A similar gradient was not observed in the control tests where no plants were present in the test solution. This gradient is thought to indicate an attachment of Pu to root surfaces and subsequent sloughing off of organic debris which settled to the bottom of the pot. This pattern indicates a preferential and rapid accumulation of Pu on the

first plant surface available which occurred during the treatment. By comparing activities of non-filtered and filtered solutions, it is obvious that most of the Pu in these samples was associated with particles. In Figure 3 the concentration of Pu remaining in solution after filtering through various-size pores is depicted. Again, the gradient decreased in the same direction as the solution flow. There was a greater abundance of particles which passed the 0.8-μm and 0.45-μm filters in the upstream samples. But apparently, all or most of the debris was larger than 0.22 μm,, and the activities passing through filters of this size probably represent soluble Pu.

When harvested, the Typha roots were placed in a cheesecloth basket and serially rinsed in various solutions. Aliquots of these rinse solutions were filtered through 0.8-μm filters, and the filters and filtrates were both analyzed for ^{238}Pu concentration. After the first water wash, 94 ± 2% of the Pu in the rinse sample was collected on the filter. After the second water wash, this had decreased to 60% and in the third washing, the filterable Pu was down to 2%. For the first two washings, summation of the activity on the filter plus that in the filtrate did not correlate well with the solution counted directly. This is attributed to self-absorption of the alpha particle in the samples with a thick layer of debris on the filter. Extremely good correlation existed in the samples subsequent to the water wash. For instance, in the citric acid solution the comparison coefficient [(nCi on the filter plus nCi in the filtrate) ÷ (nCi in unfiltered solution)] was 1.00 ± 0.05. Thus, scintillation counting is a reliable way of determining Pu concentrations in solution without particulates.

Plutonium concentration in the wash solutions after sequential washings are shown in Figures 4, 5, and 6. Each figure represents a different order of washing. The total amount of the particulate Pu removed from these root samples in all of the washings represented about 10% of the total Pu removed. Ninety-five percent of this particulate matter was removed by the first rinsing in water. Because the filterable fraction represents physical dislodgement of plant debris and the quantity was small, except in the first detergent washings, only the Pu concentration in the filtrate is presented in Figure 4, 5, and 6 as an indication of Pu release from plant surfaces.

When the washing order was water, citric acid, DTPA (Figure 4), there was maximum release of Pu into the first chelating solution. The subsequent washings each contained smaller amounts of Pu and appear to represent an ordered progression heading toward complete elution. When the order was changed to water, DTPA, citric acid, the initial release of Pu into DTPA was similar to that in Figure 4 for citric acid. The second and third washings in DTPA decreased in Pu concentration more rapidly than when citric acid was used first. Subsequent washings in citric acid released additional amounts of Pu into the solution.

Calcium chloride washes were added before elution with the two chelating agents in order to saturate the cation exchange sites with calcium presumably releasing Pu from these binding sites. The data in Figure 6 suggest that calcium chloride has little or no effect in removing additional Pu from the root tissue; subsequent washings with DTPA and citric acid gave

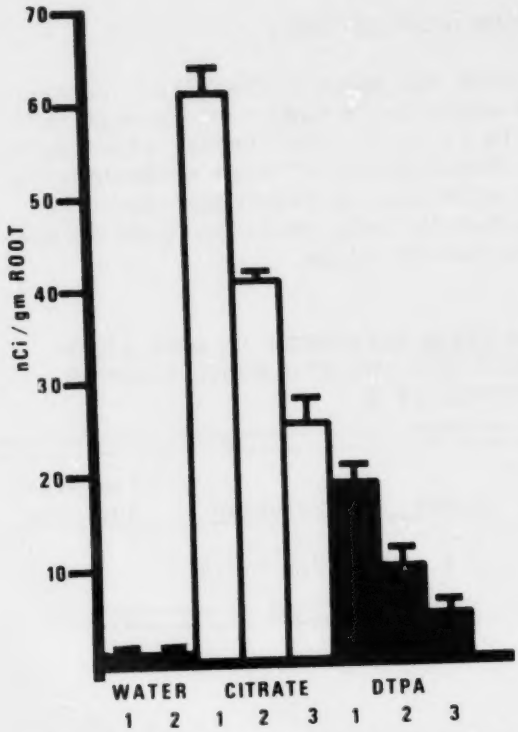

Figure 4. Plutonium in wash solutions in which Typha roots were sequentially rinsed. Washing order was: water, citric acid, DTPA. Concentration is expressed on the basis of nCi per gram of root being washed.

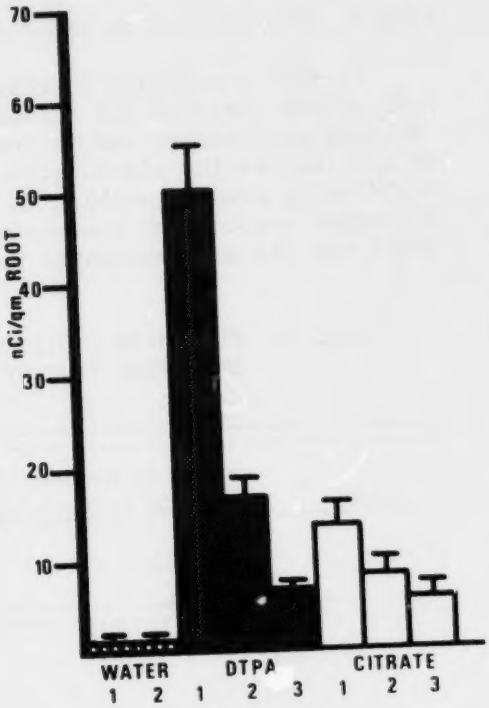

Figure 5. Plutonium in wash solutions in which Typha roots were sequentially rinsed. Washing order was: water, DTPA, citric acid. Concentration is expressed on the basis of nCi per gram of root being washed.

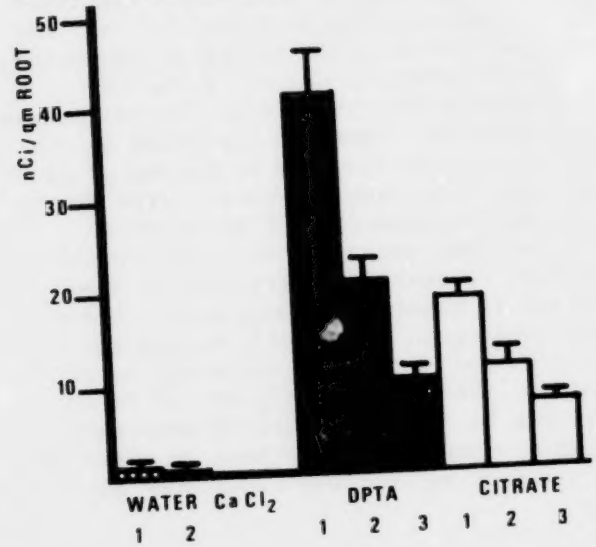

Figure 6. Plutonium in wash solutions in which Typha roots were sequentially rinsed. Washing order was: water, $CaCl_2$, DTPA, citric acid. Concentration is expressed on the basis of nCi per gram of root being washed.

results very similar to those experiments without $CaCl_2$.

At the termination of this experiment the plastic containers were sampled and analyzed for plutonium-238 content. A budget of Pu residence was then constructed and is presented in Table 4. The largest sink for Pu deposition was the plant roots. Approximately half of this contamination could be removed by washing in various solutions as previously detailed. The other segments of the system accounted for only small portions of the whole and are not considered to be important Pu sinks.

TABLE 4. PLUTONIUM DISTRIBUTION IN TYPHA EXPERIMENT 30 DAYS AFTER INJECTING PLUTONIUM NITRATE INTO THE HYDROPONIC SOLUTION EXPRESSED IN %

Roots	Activity Removed from Roots by Washing	Leaves	Solution	Container Surfaces
97.8 ± 1.8	42.3 ± 13.7	1.2 ± 1.0	0.9 ± 0.5	1.0

± = 1 standard deviation.

DISCUSSION

In this study, three plant species were grown in hydroponic solution contaminated with Pu. In all cases, Pu accumulated on the submerged plant tissues after acute or chronic exposure. A strong concentration gradient persisted between the submerged plant material and the solution. The ratio of root activity (expressed in nCi/g) to the solution activity (nCi/ml) was approximately 10^6 indicating a strong affinity of plant tissue to plutonium. When Hodge et al. (1974) studied the accumulation of fallout Pu on the Giant Brown algae off the coast of California they found that the concentration on the blades of the kelp far exceeded the concentration found in the ocean. The older blades were approximately four times as contaminated as the young blades, indicating that the length of time of contact with the contaminated seawater was important in the accumulation of Pu by this organism. This accumulation of Pu on kelp was suggested by those authors as useful in monitoring Pu concentrations in seawater. From our studies, it is clear that submerged plant tissues in general accumulate Pu. Therefore, we suggest that submerged plant tissues in any aquatic ecosystem, including fresh water, will accumulate Pu and should be considered as an appropriate monitor for Pu contamination. This strong affinity of Pu to plants might even be utilized to scavenge Pu from contaminated waterways. Plants such as water hyacinth (Eichornia crassipes) which have an extensive filamentous root system should be considered as possible candidates for such a task.

Absorption and translocation of Pu to the aerial portion of emergent aquatic species were observed to be quite low. In soil studies ratios of the Pu concentration in foliar plant tissue divided by the concentration in the rooting media (discrimination ratios) have generally been reported to vary between 10^{-3} to 10^{-6} with most of the numbers being approximately 10^{-4}. Using the concentration of Pu in the media (in this case the hydroponic culture solution) and the Pu concentration in the Typha leaves to calculate this ratio, the values were 10^{3} to 10^{5} depending on leaf age. These are clearly different than the plant:soil ratios typically reported in the literature. This example points to the importance of specifying the experimental procedures carefully in association with discussions of supposedly comparable discrimination ratios.

If discrimination ratios are recalculated in an unconventional way by using the concentration of Pu on the root compared to the concentration in the aerial portions of the plants, ratios of 10^{-4} are obtained. These are similar in magnitude to the more conventional calculation (activity of plants ÷ activity in soil) found in many soil experiments. These data seem to imply that Pu attached to the surface of the root is generally unavailable for absorption and translocation by the plant, but is only as available to uptake as is Pu in soil. It also suggests that in terms of absorption into plant tissue, close physical contact with Pu may not be of any particular value in some cases. When Jacobson and Overstreet (1948) grew barley in an aerated clay slurry, they found a positive correlation between the amount of Pu absorbed by the roots and the percent which was translocated to the leaves. They concluded that Pu fixation on the clay and roots was primarily a surface phenomenon. Although a positive correlation between root and leaf content also existed in our experiments, the large discrimination against absorption and translocation makes us believe that uptake was primarily determined by something other than the proximity of Pu to the roots. In solution culture, as in soil, solubilization or complexing of Pu to a form suitable for absorption is probably associated with exudates from roots.

When contaminated plant tissues were washed with weak detergent, no significant amount of soluble Pu was released into the solution. Since detergent would release particles held by the van der Waal forces of adhesion, these results imply that Pu was not attached to the submerged plant tissues by adhesion, but by some stronger bond. Roots typically slough off old cells and undoubtedly this is the primary source of Pu-labeled particulate matter found in sediments and washings. When roots were washed in a calcium chloride solution, no detectable quantities of Pu were removed into the solution. Calcium ions are high in the lyotropic exchange series and used in high concentrations would replace most other cations at the exchange sites on the roots. Francis (1973) reviewed the current literature regarding Pu movement in soils and, from observations that inorganic salts did not cause desorption of Pu from soils, concluded that Pu may form an anion complex with soil. Cummings and Bankert (1971) showed a lack of correlation between plant uptake of Pu and the cation exchange capacity of the substrate soil. Taken together, those authors' conclusions lead to speculation that root complexing of Pu may be due to anion bonding. Alternately, it seems possible that Pu with a large positive charge may immediately develop bonds in the plant material more stable than those formed by Ca^{++}. Cation exchange

sites on plant roots appear to be mainly carboxyl groups of the pectic substances (Keller and Deuel, 1957). No drastic change in potential for cation exchange is evident upon the death of root cells, therefore Pu remains attached to those cells when they were sloughed off.

When the alfalfa roots were washed in 0.01N nitric acid, no detectable Pu was released in this solution. Release of Pu from roots was obtained, however, in response to DTPA and to citric acid. Serial rinsing in DTPA released smaller and smaller amounts of Pu from the roots. However, subsequent rinsing in citrate caused a release of additional Pu from the roots. This may represent two forms of binding of Pu to plant roots or perhaps the existence of more than one chemical form of Pu.

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